

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently Amended): A method for constructing recombinant herpes simplex virus capable of expressing a target protein in cancer cell, comprising the steps of:

step 1: inserting into a herpes simplex virus genome, a BAC plasmid, which has a loxP site and an FRT site and into which has been inserted at least one type of marker gene expression cassette having a structure in which a marker gene is functionally linked downstream of a promoter, between the loxP site and FRT site;

step 2: constructing a shuttle vector into which has been respectively inserted at least one type of expression cassette of a gene encoding the target protein having a structure in which the gene encoding the target protein is functionally linked downstream of a promoter, at least one type of marker gene, a loxP site and an FRT site, wherein said shuttle vector contains a stuffer sequence, and inserting said shuttle vector into the loxP site of the herpes simplex virus genome using Cre recombinase so as to realize a constitution which allows expression of the gene encoding the target protein and the marker gene, wherein the herpes simplex virus genome is about 168k nucleotides or larger after the insertion of the shuttle vector, exclusive of the gene encoding the target protein; or wherein the herpes simplex virus genome is about 170k nucleotides or larger after the insertion of the shuttle vector, including the nucleotides encoding the target protein; and,

step 3: co-infecting a host with the herpes simplex virus genome obtained in the second step and a vector capable of expressing Flp recombinase, and excising the region between the FRT sites in said genome to produce a target recombinant herpes simplex virus that is at least 150k nucleotides.

2. (original): The method according to claim 1, wherein the second step is carried out in a liquid phase.

3. (original): The method according to claim 1 or claim 2, wherein a γ 34.5 gene and ICP6 gene of the herpes simplex virus are deleted or inactivated prior to the first step.

4. (original): The method according to claim 3, wherein ICP47 gene of the herpes simplex virus is additionally deleted or inactivated.

5. (Previously Presented): The method according to claim 1, wherein the marker gene inserted into the BAC plasmid is a gene encoding green fluorescent protein (GFP) and/or an antibiotic resistance gene.

6. (Previously Presented): The method according to claim 1, wherein the promoter contained in at least one type of expression cassette of a gene encoding the target protein is a promoter comprising a nucleotide sequence not present in the naturally-occurring herpes simplex virus genome.

7. (Previously Presented): The method according to claim 1, wherein the promoter comprising a nucleotide sequence not present in the herpes simplex virus genome is CMV promoter.

8. (Previously Presented): The method according to claim 1, wherein the marker gene inserted into the shuttle vector is lacZ gene and/or an antibiotic resistance gene.

9. (original): The method according to claim 8, wherein the marker gene inserted into the shuttle vector is an antibiotic resistance gene different from the antibiotic resistance gene inserted into the BAC plasmid.

10. (Previously Presented): The method according to claim 1, wherein the gene encoding the target protein is one or more genes selected from the group consisting of an immunostimulatory gene, a anti-angiogenesis gene, a gene encoding a cell membrane fusion protein, and a tumor suppressor gene.

11. (original): The method according to claim 10, wherein the immunostimulatory gene is a gene encoding one or more proteins selected from the group consisting of co-stimulatory factor, IL-12, IL-18, IL-23, IL-27 and transporter associated with antigen processing (TAP).

12. (Withdrawn): The method according to claim 10, wherein the anti-angiogenesis gene is a gene encoding one or more proteins selected from the group consisting of endostatin, angiostatin, dominant negative FGF receptor and platelet factor 4.

13. (Withdrawn): The method according to claim 10, wherein the gene encoding a cell membrane fusion protein is a virus surface protein.

14. (Withdrawn): The method according to claim 10, wherein the tumor suppressor gene is p53 gene.

15. (Canceled)

16. (Withdrawn): The method according to claim 14, wherein the stuffer sequence is about 5000 nucleotides or more in length.

17. (Withdrawn): A recombinant herpes simplex virus constructed according to the method in claim 1.

18. (Withdrawn): A pharmaceutical composition containing a recombinant herpes simplex virus according to claim 17.

19. (Withdrawn): The pharmaceutical composition according to claim 18, which is therapeutic or preventative of various cancer diseases.

20. (Withdrawn): A method preventing or treating cancer, comprising administration of the pharmaceutical composition according to claim 18.

21. (New): The method of claim 1, wherein the target recombinant herpes simplex virus of step 3 is between about 150k nucleotides and about 170k nucleotides including the nucleotides encoding the target protein.

22. (New): The method of claim 1, wherein the target recombinant herpes simplex virus of step 3 is between about 150k nucleotides and about 168k nucleotides exclusive of the gene encoding the target protein.